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Pathogenesis of type 2 diabetes mellitus

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Normal glucose homeostasis

A discussion of the pathogenesis of type 2 diabetes mellitus must start with a review of mechanisms involved in the maintenance of normal glucose homeostasis in the basal or postabsorptive state (10–12 h overnight fast) and following ingestion of a typical mixed meal [1–9]. In the postabsorptive state the majority of total body glucose disposal takes place in insulin-independent tissues. Thus, approximately 50% of all glucose use occurs in the brain, which is insulin-independent and becomes saturated at a plasma glucose concentration of approximately 40 mg/dL [10]. Another 25% of glucose disposal occurs in the splanchnic area (liver plus gastrointestinal tissues), which is also insulin-independent. The remaining 25% of glucose use in the postabsorptive state takes place in insulin-dependent tissues, primarily muscle, and to a lesser extent adipose tissue. Basal glucose use, approximately 2.0 mg/kg/min, is precisely matched by the rate of endogenous glucose production (Fig. 1). Approximately 85% of endogenous glucose production is derived from the liver, and the remaining 15% is produced by the kidney. Glycogenolysis and gluconeogenesis contribute equally to the basal rate of hepatic glucose production.

Following glucose ingestion, the increase in plasma glucose concentration stimulates insulin release, and the combination of hyperinsulinemia and hyperglycemia (1) stimulates glucose uptake by splanchnic (liver and gut) and peripheral (primarily muscle) tissues and (2) suppresses endogenous (primarily hepatic) glucose production (Box 1) [1–9].

The majority (~80%–85%) of glucose uptake by peripheral tissues occur in muscle, with a small amount (~4%–5%) metabolized by adipocytes. Although fat tissue is responsible for only a small amount of total body glucose disposal, it plays a very important role in the maintenance of total body glucose homeostasis by regulating the release of free fatty acids (FFA) from stored triglycerides (see discussion below) and through the production of adipocytokines that influence insulin sensitivity in muscle and liver

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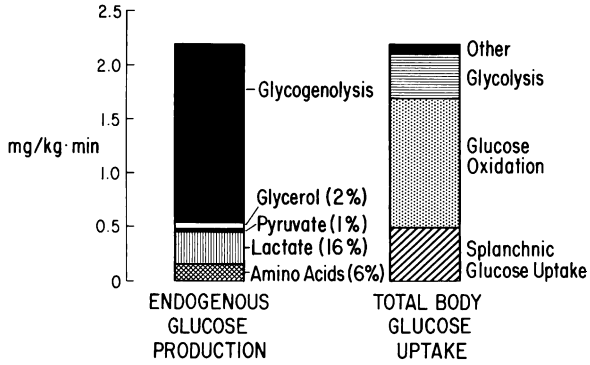

Fig. 1. Postabsorptive state. Glucose production and glucose use in the normal human in the postabsorptive state. (*From* DeFronzo RA. Pathogenesis of type 2 diabetes mellitus: metabolic and molecular implications for identifying diabetes genes. Diabetes 1997;5:117–9; with permission.)

[11–14]. Insulin is a potent antilipolytic hormone, and even small increments in the plasma insulin concentration markedly inhibit lipolysis, leading to a decline in the plasma level of free fatty acid [12]. The decline in plasma FFA concentration augments muscle glucose uptake and contributes to the inhibition of hepatic glucose production. Thus, changes in the plasma FFA concentration in response to increased plasma levels of insulin and glucose play an important role in the maintenance of normal glucose homeostasis [11–14].

Glucagon also plays a central role in the regulation of glucose homeostasis [9,15]. Under postabsorptive conditions, approximately half of total hepatic glucose output is dependent on the maintenance of normal basal glucagon levels, and inhibition of basal glucagon secretion with somatostatin causes a reduction in hepatic glucose production and plasma

Box 1. Factors responsible for the maintenance of normal glucose tolerance in healthy subjects

A. Insulin secretion
B. Tissue glucose uptake
   1. Peripheral (primarily muscle)
   2. Splanchnic (liver plus gut)
C. Suppression of HGP
   1. Decreased FFA
   2. Decreased glucagons
D. Route of glucose administration

glucose concentration. After a glucose-containing meal, glucagon secretion is inhibited by hyperinsulinemia, and the resultant hypoglucagonemia contributes to the suppression of hepatic glucose production and maintenance of normal postprandial glucose tolerance.

The route of glucose entry into the body also plays an important role in the distribution of administered glucose and overall glucose homeostasis [9,16,17]. Intravenous insulin exerts only a small stimulatory effect on splanchnic (liver plus gut) glucose uptake, whereas intravenous glucose augments splanchnic glucose uptake in direct proportion to the increase in plasma glucose concentration. In contrast, oral glucose administration markedly enhances splanchnic glucose uptake. The portal signal that stimulates hepatic glucose uptake after glucose ingestion is directly proportional to the negative hepatic artery–portal vein glucose concentration gradient [9]. As this gradient widens, the splanchnic nerves are stimulated, and this activates a neural reflex in which vagal activity is enhanced, and sympathetic nerves innervating the liver are inhibited. These neural changes augment liver glucose uptake and stimulate hepatic glycogen synthase, while simultaneously inhibiting glycogen phosphorylase. In contrast to intravenous glucose/insulin administration, in which muscle accounts for the majority (~80%–85%) of glucose disposal, the splanchnic tissues are responsible for the removal of approximately 30%–40% of an ingested glucose load. Glucose administration through the gastrointestinal tract also has a potentiating effect on insulin secretion. Thus, the plasma insulin response following oral glucose is approximately twice as great as that following intravenous glucose, despite equivalent increases in the plasma glucose concentration. This increase in effect is related to the release of glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP) (previously called gastric inhibitory polypeptide) from the intestinal tissues cells [18,19].

Glucose homeostasis in type 2 diabetes mellitus

Type 2 diabetic subjects manifest multiple disturbances in glucose homeostasis, including: (1) impaired insulin secretion; (2) insulin resistance in muscle, liver, and adipocytes; and (3) abnormalities in splanchnic glucose uptake [1,2,20,21].

Insulin secretion in type 2 diabetes mellitus

Impaired insulin secretion is found uniformly in type 2 diabetic patients in all ethnic populations [1,2,20–29]. Early in the natural history of type 2 diabetes, insulin resistance is well established but glucose tolerance remains normal because of a compensatory increase in insulin secretion. This dynamic interaction between insulin secretion and insulin resistance has been well documented. Within the normal glucose tolerant population, approximately

20%–25% of individuals are severely resistant to the stimulatory effect of insulin on glucose uptake (Fig. 2) (measured with the euglycemic insulin clamp), and subjects in the lowest quartile of insulin sensitivity are as insulin resistant as type 2 diabetics (see Fig. 2). Insulin secretion (measured with the hyperglycemic clamp technique) in these insulin-resistant, nondiabetic individuals, however, is increased in proportion to the severity of the insulin resistance, and glucose tolerance remains normal. Thus, the beta cells are able to "read" the severity of insulin resistance and adjust their secretion of insulin to maintain normal glucose tolerance.

In type 2 diabetics, the fasting plasma insulin concentration is normal or increased and basal insulin secretion (measured from C-peptide kinetics) is elevated. The relationship between the fasting plasma glucose (FPG) and

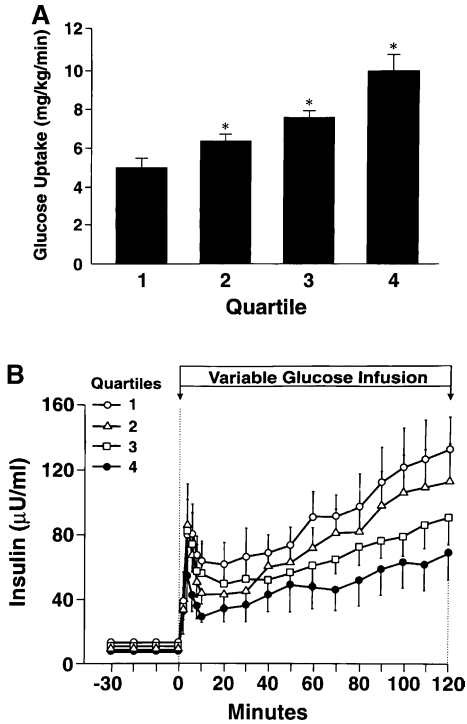

Fig. 2. (*A*) Whole-body rate of glucose disposal during euglycemic insulin clamps in 32 women divided according to quartiles of insulin sensitivity. * $P \leq 0.001$ for each quartile versus the adjacent quartile. (*B*) Time course of plasma insulin response during the hyperglycemic clamp in the same 32 women divided into quartiles of insulin sensitivity. Insulin secretion rose progressively from the highest to the lowest quartile of insulin sensitivity ($P \leq 0.01$). ○, Quartile 1; △, quartile 2; □, quartile 3; ●, quartile 4. (*From* Diamond MP, Thornton K, Connolly-Diamond M, Sherwin RS, DeFronzo RA. Reciprocal variations in insulin-stimulated glucose uptake and pancreatic insulin secretion in women with normal glucose tolerance. J Soc Gynecol Invest 1995;2:708–15.)

insulin concentrations resembles an inverted U shape or horseshoe [1,2]. Because this curve resembles Starling's curve of the heart, it has been referred to as Starling's curve of the pancreas. As the fasting glucose rises from 80 to 140 mg/dL, the fasting plasma insulin concentration increases progressively, reaching a peak value 2.0–2.5-fold greater than in normal weight, nondiabetic, age-matched controls. The progressive rise in fasting plasma insulin level can be viewed as an adaptive response of the pancreas to offset the progressive deterioration in glucose homeostasis. When the FPG exceeds 140 mg/dL, the beta cell is unable to maintain its elevated rate of insulin secretion, and the fasting insulin concentration declines precipitously. This decrease in fasting insulin level has important physiologic implications, because it is at this point that hepatic glucose production (the primary determinant of the FPG concentration) begins to rise.

The relationship between the mean plasma insulin response during an OGTT and the FPG concentration also resembles an inverted U-shaped curve (Fig. 3) [1,2]. The curve, however, is shifted to the left compared with the basal insulin secretory rate, and the glucose-stimulated insulin response begins to decline at a fasting glucose concentration of approximately 120 mg/dL. A typical type 2 diabetic subject with a FPG level of 150–160 mg/dL secretes an amount of insulin similar to that in a healthy nondiabetic individual; however, a "normal" insulin response in the presence of hyperglycemia and underlying insulin resistance is markedly abnormal. At FPG levels in excess of 150–160 mg/dL, the plasma insulin response, when viewed in absolute terms, becomes

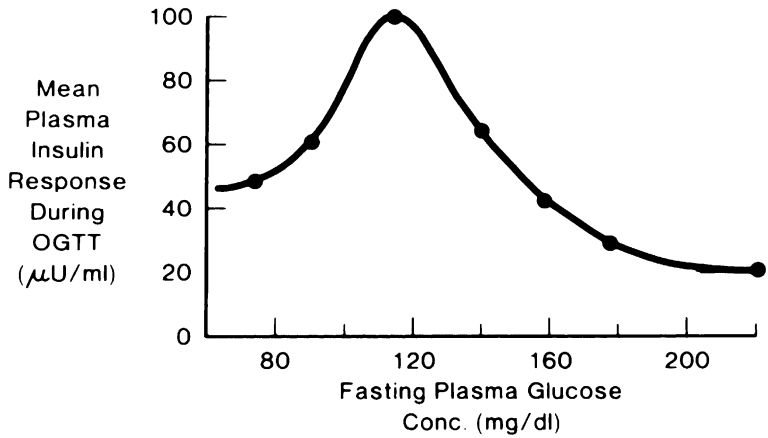

Fig. 3. Starling's curve of the pancreas for insulin secretion. In normal-weight patients with IGT and mild diabetes, the plasma insulin response to OGTT increases progressively until the fasting glucose reaches 120 mg/dL. Thereafter, further increases in the fasting glucose concentration are associated with a progressive decline in insulin secretion. (*From* DeFronzo RA. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. Diabetes 1988;37(6):667–87; with permission.)

insulinopenic. Finally, when the fasting glucose exceeds 200–220 mg/dL, the plasma insulin response to a glucose challenge is markedly blunted. Nonetheless, the fasting hyperinsulinemia persists despite FPG concentrations as high as 250–300 mg/dL, and 24-hour integrated plasma insulin and C-peptide profiles in lean type 2 diabetic patients remain normal. These normal day-long values result from the combination of elevated fasting and decreased postprandial insulin and C-peptide secretory rates [30,31].

It should be emphasized that, even though the plasma insulin response is increased in absolute terms early in the development of type 2 diabetes (FPG $\leq$ 140 mg), this does not mean that beta-cell function is normal. The beta cell responds to an increment in plasma insulin (ΔI) by an increment in plasma glucose (ΔG) and this response is modulated by the severity of insulin resistance, that is, the more severe the insulin resistance, the greater the insulin response. When this index of beta-cell function is plotted against the 2-hour plasma glucose concentration during the OGTT, the loss of 60%–70% of beta-cell function can be appreciated in individuals with impaired glucose tolerance. In fact, normal glucose tolerant individuals in the upper tertile of glucose tolerance (2-h plasma glucose, 120–140 mg/dL) already have lost 50% of their beta-cell function [32].

The natural history of type 2 diabetes, starting with normal glucose tolerance, insulin resistance, and compensatory hyperinsulinemia, with progression to impaired glucose tolerance (IGT) and overt diabetes mellitus, has been observed in a variety of populations including whites, Native Americans, Mexican Americans, and Pacific Islanders, and in the rhesus monkey, an animal model that closely resembles type 2 diabetes in humans [1, 2,20–28,33–35]. These population studies have demonstrated a strong association between obesity and type 2 diabetes, leading to the new-world syndrome of "diabesity." In high-risk populations, the progression from normal to IGT is associated with marked increases in both fasting and glucose-stimulated plasma insulin levels and a decrease in tissue sensitivity to insulin (Fig. 4). The progression from IGT to type 2 diabetes with mild fasting hyperglycemia (120–140 mg/dL, 6.7–7.8 mmol/L) is heralded by an inability of the beta cell to maintain its previously high rate of insulin secretion in response to a glucose challenge without further or only minimal deterioration in tissue sensitivity to insulin. Increased basal insulin secretion and fasting hyperinsulinemia, however, are maintained until the FPG exceeds 140 mg/dL. A similar pattern of insulin secretion has been observed during the development of diabetes in the rhesus monkey [33]. The aging monkey becomes obese, and a high percentage of monkeys develop typical type 2 diabetes. The earliest detectable abnormality (preceding the onset of diabetes mellitus) is a decrease in tissue sensitivity to insulin, with a compensatory increase in fasting and glucose-stimulated plasma insulin concentrations. With time, the high rate of insulin secretion cannot be maintained, the beta cell starts on downward slope of Starling's curve (see Fig. 3), and fasting hyperglycemia and glucose intolerance ensue. In summary, these studies are consistent in demonstrating

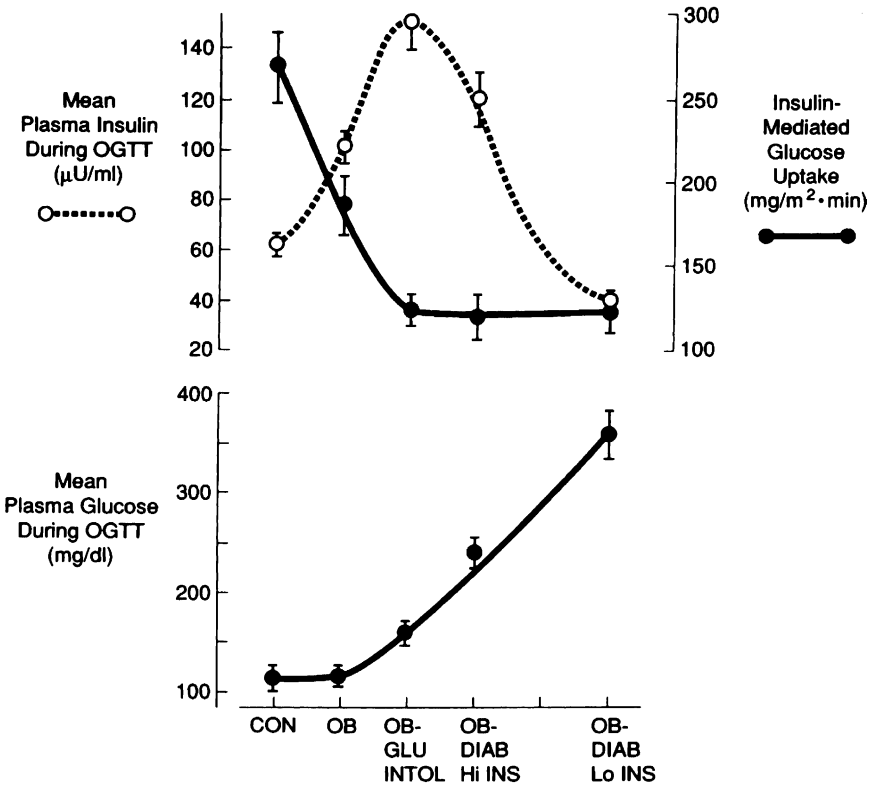

Fig. 4. Summary of the plasma insulin (*top*, ○) and plasma glucose (*bottom*, ●) responses during a 100-g OGTT and tissue sensitivity to insulin (*top*, ●) in control (CON), obese nondiabetic (OB), obese glucose intolerant (OB-GLU INTOL), obese hyperinsulinemic diabetic (OB-DIAB Hi INS), and obese hypoinsulinemic diabetic subjects (OB-DIAB Lo INS). See text for a detailed discussion. (*From* DeFronzo RA. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. Diabetes 1988;37(6):667–87; with permission.)

that hyperinsulinemia precedes the development of type 2 diabetes, and hyperinsulinemia is a strong predictor of the development of IGT and type 2 diabetes. It should be emphasized, however, that overt diabetes (fasting glucose $\geq$ 126 mg/dL) does not develop in the absence of a significant defect in beta-cell function. The nature of this beta-cell defect is considered in subsequent sections.

Type 2 diabetes with hypoinsulinemia

A large body of clinical and experimental evidence documents that hyperinsulinemia and insulin resistance precede the onset of type 2 diabetes. Nonetheless, a number of studies have shown that absolute insulin deficiency, with or without impaired tissue insulin sensitivity, can lead to the development

of type 2 diabetes. This scenario is best exemplified by patients with maturity onset diabetes of youth (MODY) [36–38]. This familial subtype of type 2 diabetes is characterized by early age of onset, autosomal dominant inheritance with high penetrance, mild to moderate fasting hyperglycemia, and impaired insulin secretion.

MODY originally was described by Fajans and coworkers [39], and subsequently it was demonstrated that MODY1 resulted from a nonsense mutation in exon 7 of the hepatic nuclear factor (*HNF*)*4*α gene. It later was demonstrated that the occurrence of MODY in French families resulted from mutations in the glucokinase gene on chromosome 7p (MODY2) [40]. Six specific mutations in different genes have been implicated in the MODY profile, including glucokinase and five transcription factors [36–40]: MODY1, *HNF4*α; MODY2, glucokinase; MODY3, *HNF1*α; MODY4, insulin promoter factor 1; MODY5, *HNF1*β; and MODY6, neurogenic differentiation 1/beta-cell E-box transactivator 2. The hallmark defect in MODY individuals is impaired insulin secretion in response to glucose and other secretagogues; however, peripheral tissue resistance to insulin and abnormalities in hepatic glucose metabolism also have been shown to play some role in the development of impaired glucose homeostasis [41,42]. Although glucokinase mutations are characteristic of MODY2, genetic studies in typical older-onset type 2 diabetic individuals have shown that glucokinase mutations account for less than 1% of the common form of type 2 diabetes [43].

Cerasi [21] and Luft [44] and Hales [45] and coworkers proposed that insulin deficiency represents the primary defect responsible for glucose intolerance in typical type 2 diabetics who do not have glucokinase or other MODY mutations. According to these investigators, impaired early insulin secretion leads to an excessive rise in plasma glucose concentration, and the resultant hyperglycemia is responsible for late hyperinsulinemia. Hales and colleagues [45] have demonstrated that many lean whites with mild fasting hyperglycemia ($\leq$140 mg/dL, 7.8 mmol/L) are characterized by insulin deficiency at all time points during an OGTT. An impaired early insulin response also has been a characteristic finding in Japanese Americans who progress to type 2 diabetes [46]. Unfortunately, none of these studies provide information about insulin sensitivity. In whites, several investigating groups [47,48] have demonstrated normal insulin sensitivity in a minority of type 2 diabetic individuals, and it has been suggested that up to 50% of African-American type 2 diabetic patients who reside in New York City are characterized by severely impaired insulin secretion and normal insulin sensitivity [49]. A similar defect in insulin secretion has been described in black African type 2 diabetics living in Cameroon [50]. In summary, it is clear that impaired insulin secretion, in the absence of insulin resistance, can lead to the development of full-blown type 2 diabetes, but it remains to be clarified how frequently a pure beta-cell defect results in typical type 2 diabetes in the general population.

First-phase insulin secretion

In response to intravenous glucose, insulin is secreted in a biphasic pattern, with an early burst of insulin release within the first 10 minutes followed by a progressively increasing phase of insulin secretion that persists as long as the hyperglycemic stimulus is present [51]. This biphasic insulin response is not observed after taking oral glucose because of the more gradual rise in plasma glucose concentration. Loss of first-phase insulin secretion is a characteristic and early abnormality in patients destined to develop type 2 diabetes. In most type 2 diabetic subjects, a reduction in the early phase of insulin secretion during the OGTT (0–30 min) and during the intravenous glucose tolerance test (0–10 min) becomes evident when FPG concentration exceeds 110–120 mg/dL (6.1–6.7 mmol/L) [34,35,51–53]. During the OGTT, the defect in early insulin secretion is most obvious if the incremental plasma insulin response at 30 min is expressed relative to the incremental plasma glucose response at 30 min ($\Delta I_{30} \div \Delta G_{30}$). Although the first-phase insulin secretory response to intravenous glucose characteristically is diminished or lost in type 2 diabetes, this defect is not consistently observed until the FPG concentration rises to approximately115–120 mg/dL (6.4–6.7 mmol/L). The defect in first-phase insulin response can be partially restored with tight metabolic control [54,55], indicating that at least part of the defect is acquired, most likely secondary to glucotoxicity or liptoxicity [11,56–59] (see subsequent discussion). Loss of the first phase of insulin secretion has important pathogenic consequences, because this early burst of insulin primes insulin target tissues, especially the liver, that are responsible for the maintenance of normal glucose homeostasis [60,61].

Causes of impaired insulin secretion in type 2 diabetes mellitus

Progression from normal glucose tolerance to IGT to type 2 diabetes with mild fasting hyperglycemia ($\leq$120–140 mg/dL, 6.7–7.8 mmol/L) is characterized by hyperinsulinemia (see Figs. 3 and 4) [1,2,32]. When the fasting glucose concentration exceeds approximately 120 mg/dL (6.7 mmol/L) and approximately140 mg/dL (7.8 mmol/L), respectively, the fasting and glucose-stimulated plasma insulin levels decline progressively. A number of pathogenic genetic and acquired factors have been implicated in the progressive impairment in insulin secretion. Pancreatic beta cells are in a constant state of dynamic change, with continued regeneration of islets from ductal endothelial cells of the exocrine pancreas and simultaneous apoptosis [62]. Multiple abnormalities have been shown to disturb the delicate balance between islet neogenesis and apoptosis (see subsequent discussion).

Studies in first degree relatives of type 2 diabetic patients and in twins have provided strong evidence for the genetic basis of beta-cell dysfunction [63–66]. Impaired insulin secretion also has been shown to be an inherited trait in Finnish families with type 2 diabetes mellitus with evidence for a susceptibility locus on chromosome 12 [67].

Both "glucotoxicity" [2,56] and "lipotoxicity" [11,57–59] are among the acquired defects that can lead to impaired insulin secretion (Fig. 5). The glucotoxicity hypothesis is supported by the observation that improved glycemic control, however it is achieved (diet, insulin therapy, sulfonylureas, metformin), leads to enhanced insulin secretion [54,55]. More direct support of the glucotoxicity hypothesis comes from animal studies in which diabetic rats were treated with phlorizin, a potent renal tubular glucose transporter inhibitor that reduces the plasma glucose concentration without altering other circulating substrate levels [68]. When administered to partially pancreatectomized, chronically hyperglycemic diabetic rats, phlorizin restores normoglycemia and results in a dramatic improvement in both first- and second-phase insulin secretion. Conversely, when nondiabetic rats with a reduced beta-cell mass are exposed in vivo to a chronic physiologic increment in plasma glucose concentration of as little as 15 mg/dL, insulin secretion by the pancreas perfused in vitro is inhibited by 75% [69,70]. These provocative results suggest that a minimal elevation in mean plasma glucose concentration, in the presence of a reduced beta-cell mass, can lead to a major impairment in insulin secretion by the remaining pancreatic tissue. Prolonged beta cell exposure to high glucose concentrations in vitro also has been shown to impair insulin gene transcription, leading to decreased insulin synthesis and secretion [71].

Lipotoxicity [11,57–59,72] also has been implicated as an acquired cause of impaired beta-cell function, as individuals progress from IGT to overt type 2 diabetes mellitus. Short-term exposure of beta cells to physiologic increases in free fatty acids stimulates insulin secretion. Within the beta cell, long-chain fatty acids are converted to their fatty acyl-CoA derivatives, which lead to increased formation of phosphatidic acid and diacylglycerol. These lipid intermediates activate specific protein kinase C isoforms, which enhances the exocytosis of insulin. Long-chain fatty acyl-CoA also stimulate

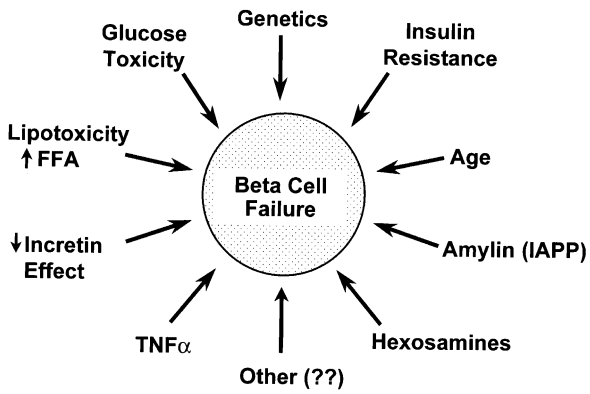

Fig. 5. Pathogenetic factors implicated in the progressive impairment in insulin secretion in type 2 diabetes mellitus. TNFα, tumor necrosis factor-α.

exocytosis, cause closure of the K^+-ATPase channel, stimulate Ca^{2+}-ATPase and increase intracellular calcium, thus augmenting insulin secretion. In contrast to these acute effects, chronic beta cell exposure to elevated fatty acyl-CoA inhibits insulin secretion through operation of the Randle cycle. Increased fatty acyl-CoA levels within the beta cells also stimulate ceramide synthesis, which augments inducible nitric-oxide synthase. The resultant increase in nitric oxide increases the expression of inflammatory cytokines, including interleukin-1 and tumor necrosis factor α, which impair beta-cell function and promote beta cell apoptosis.

Most recently, deficiency of or resistance to "incretins" have been implicated in the pathogenesis of beta-cell dysfunction in type 2 diabetic patients [18,19,73–78]. When glucose is administered through the gastrointestinal route, a much greater stimulation of insulin secretion is observed compared with a similar level of hyperglycemia created with intravenous glucose [73]. This observation prompted a search for the responsible "incretins" or gut-derived hormones that enhance glucose-stimulated insulin secretion following the oral route of glucose administration. Two gastrointestinal hormones, GIP and GLP-1, have been shown to account for more than 90% of the incretin effect observed following glucose or mixed-meal ingestion [74–78]. Both GIP and GLP-1 are released from endocrine cells of the duodenum and jejunum in response to intraluminal carbohydrate but not in response to circulating glucose. The stimulation of insulin secretion by both GIP and GLP-1 is dependent on the ambient glucose concentration, which is greater when plasma glucose concentration is high and absent when the plasma glucose concentration returns to basal levels. Antibodies that neutralize GIP and GLP-1 impair glucose tolerance in a variety of animal species, including primates. Although the amount of GLP-1 released is considerably less than that of GIP, GLP-1 is such a potent potentiator of insulin secretion that it is thought to be the major incretin. In type 2 diabetic humans, the GIP response to glucose ingestion is normal, indicating the presence of beta-cell resistance to the incretin-effect of GIP. In contrast, the GLP-1 response to oral glucose is reduced. Acute intravenous administration of GLP-1 in type 2 diabetic patients enhances the postprandial insulin secretory response, and chronic continuous GLP-1 administration restores near-normal glycemia in type 2 diabetic patients [74,79]. GLP-1 also has been shown to augment islet regeneration in rodents [80].

Amylin (islet amyloid polypeptide [IAPP]) has been implicated in progressive beta-cell failure in type 2 diabetes mellitus [81–83]. IAPP, which is packaged with insulin in secretory granules and co-secreted into the sinusoidal space, is the precursor for the amyloid deposits that are frequently observed in type 2 diabetic and occur spontaneously in diabetic monkeys. At very high doses, amylin has been shown to inhibit insulin secretion by the perfused rat pancreas in vitro. Elevated plasma islet amyloid polypeptide levels have been demonstrated in type 2 diabetic subjects, obese glucose-intolerant subjects, glucose-intolerant first-degree

relatives of type 2 diabetic patients, and in animal models of diabetes [81–83]. Following its secretion, amylin accumulates extracellularly in close proximity to the beta cell, and it has been suggested that amylin deposits cause beta-cell dysfunction. Although it is attractive, this theory has been challenged by Bloom and coworkers [84], who failed to find any inhibitory effect of amylin on insulin secretion when the peptide was infused in pharmacologic doses in rats, rabbits, and humans. Studies in transgenic mice [85], which express the gene encoding either human or rat IAPP under control of an insulin promoter, also mitigate against an important role of IAPP in the development of beta-cell dysfunction. Thus, although pancreatic and plasma amyloid polypeptide levels were significantly elevated in these transgenic mice, hyperglycemia and hyperinsulinemia did not develop. A recent provocative review [86] even suggests that IAPP in the islets of Langerhans may serve a protective role under conditions of increased insulin secretion. In summary, definitive evidence that amylin contributes to beta-cell dysfunction in human type 2 diabetes remains elusive, although recent evidence [81] suggests that the combination of elevated plasma FFA levels and amylin hypersecretion may interact synergistically to impair insulin secretion and cause beta-cell injury.

The number of beta cells within the pancreas is an important determinant of the amount of insulin that is secreted. Most [87–90] but not all [91,92] studies have demonstrated a modest reduction (20%–40%) in beta-cell mass in patients with long-standing type 2 diabetes. Obesity, another insulin-resistant state, is characterized by a significant increase in beta-cell mass [88], and the majority of type 2 diabetics are overweight. Thus, even a modest reduction (20%–40%) in beta-cell mass is most impressive. On routine histologic examination, the islets of Langerhans appear normal with the exception of beta-cell degranulation [87–90]. Insulitis is not observed. The factors responsible for the decrease in beta-cell mass in type 2 diabetics remain to be identified. Several studies suggest that new islet formation from exocrine ducts is reduced in type 2 diabetic individuals [93]. In animal model of diabetes, there is evidence that islet neogenesis is reduced and beta-cell apoptosis is accelerated [94]. Although recent studies with well-matched controls (age, gender, and obesity) suggest that beta-cell mass is reduced, even during the early stages of the development of type 2 diabetes, it seems likely that factors in addition to beta-cell loss must be responsible for the impairment in insulin secretion.

Low birth weight is associated with the development of IGT and type 2 diabetes in a number of populations [95]. Developmental studies in animals and humans have demonstrated that poor nutrition and impaired fetal growth (small babies at birth) are associated with impaired insulin secretion or reduced beta-cell mass. Fetal malnutrition also can lead to the development of insulin resistance later in life [96]. One could hypothesize that an environmental influence, for example, impaired fetal nutrition leading to an acquired defect in insulin secretion or reduced beta-cell mass, when

superimposed on insulin resistance, could eventuate in type 2 diabetes later in life. Thus, during the normal aging process, with the onset of obesity or with a worsening of the genetic component of the insulin resistance, the beta cell would be called on to augment its secretion of insulin to offset the defect in insulin action. If beta-cell mass (or function) is reduced (or impaired) by an environmental insult during fetal life, this would lead to the development of IGT and eventually to overt type 2 diabetes. Although such a defect would limit the maximum amount of insulin that could be secreted, it would not explain the progressive decline in insulin secretion in response to physiological stimuli as individuals progress from IGT to type 2 diabetes (see Fig. 4).

Insulin resistance and type 2 diabetes

In cross-sectional studies and long-term, prospective longitudinal studies, hyperinsulinemia has been shown to precede the onset of type 2 diabetes in all ethnic populations with a high incidence of type 2 diabetes [1,2,20–27,34,35,97–102]. Studies using the euglycemic insulin clamp, minimal model, and insulin suppression techniques have provided direct quantitative evidence that the progression from normal to impaired glucose tolerance is associated with the development of severe insulin resistance, whereas plasma insulin concentrations, both in the fasting state and in response to a glucose load (see Figs. 3 and 4) are increased when viewed in absolute terms (see above discussion of insulin secretion). It should be emphasized, however, that, even though the absolute insulin secretory rate is increased, beta-cell sensitivity to glucose is markedly reduced in individuals with IGT.

Himsworth and Kerr [103], using a combined oral glucose and intravenous insulin tolerance test, were the first to demonstrate that tissue sensitivity to insulin is diminished in type 2 diabetic patients. In 1975, Reaven and colleagues [104], using the insulin suppression test, provided further evidence that the ability of insulin to promote tissue glucose uptake in type 2 diabetes was severely reduced. A defect in insulin action in type 2 diabetes also has been demonstrated with the arterial infusion of insulin into the brachial artery (forearm muscle) and femoral artery (leg muscle) as well as with radioisotope turnover studies, the frequently sampled intravenous glucose tolerance test, and the minimal model technique [1,2,5,105–107].

DeFronzo et al [1,2,5,12,105,108,109], using the more physiologic euglycemic insulin clamp technique, have provided the most conclusive documentation that insulin resistance is a characteristic feature of lean, as well as obese, type 2 diabetic individuals. Because diabetic patients with severe fasting hyperglycemia ($\geq$180–200 mg/dL, 10.0–11.1 mmol/L) are insulinopenic (see Fig. 3), and because insulin deficiency is associated with the emergence of a number of intracellular defects in insulin action, these initial studies focused on diabetics with mild to modest elevations in the FPG concentration (mean, 150 $\pm$ 8 mg/dL, 8.3 $\pm$ 0.4 mmol/L). Insulin-mediated whole-body glucose

disposal in these lean diabetics was reduced by approximately 40%–50%, providing conclusive proof of the presence of moderate to severe insulin resistance. Three additional points are noteworthy: (1) lean type 2 diabetics with more severe fasting hyperglycemia (198 ± 10 mg/dL) have a severity of insulin resistance that only is slightly greater (10%–20%) than diabetics with mild fasting hyperglycemia; (2) the defect in insulin action is observed at all plasma insulin concentrations, spanning the physiologic and pharmacologic range (Fig. 6); and in (3) diabetic patients with overt fasting hyperglycemia even maximally stimulating plasma insulin concentrations (under euglycemic conditions) cannot elicit a normal glucose metabolic response. With a few exceptions, the majority of investigators have demonstrated that lean type 2 diabetic subjects are resistant to the action of insulin [24–27,29,34,35,97,101,107,110–112]. The ability of glucose (hyperglycemia) to stimulate its own uptake, that is, the mass action effect of hyperglycemia, also is impaired in type 2 diabetics [113].

Site of insulin resistance in type 2 diabetes

Maintenance of normal whole-body glucose homeostasis requires a normal insulin secretory response and normal tissue sensitivity to the independent effects of hyperinsulinemia and hyperglycemia to augment glucose uptake [1–7]. The combined effects of insulin and hyperglycemia to promote glucose disposal are dependent on three tightly coupled mechanisms (see Box 1): (1) suppression of endogenous (primarily hepatic) glucose production; (2) stimulation of glucose uptake by the splanchnic

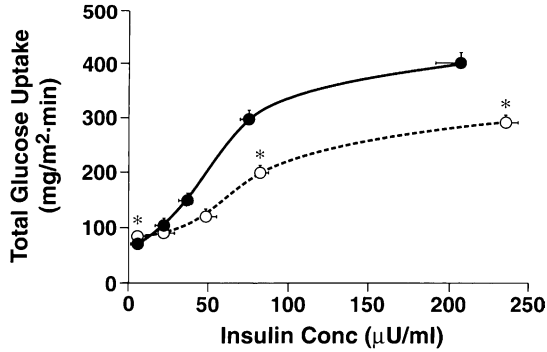

Fig. 6. Dose-response curve relating the plasma insulin concentration to the rate of insulin-mediated whole-body glucose uptake in control (●) and type 2 diabetic (○) subjects. * $P \leq 0.01$ versus control subjects. (*From* Groop LC, Bonadonna RC, DelPrato S, Ratheiser K, Zyck K, Ferrannini E, DeFronzo RA. Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. J Clin Invest 1989;84(1):205–13; with permission.)

(hepatic plus gastrointestinal) tissues; and (3) stimulation of glucose uptake by peripheral tissues, primarily muscle. Muscle glucose uptake is regulated by flux through two major metabolic pathways: glycolysis (of which ~90% represents glucose oxidation) and glycogen synthesis.

Hepatic glucose production

In the postabsorptive state, the liver of healthy subjects produces glucose at the rate of 2.0 mg/kg/min [1,2,12,114]. This glucose efflux is essential to meet the needs of the brain and other neural tissues, which use glucose at a constant rate of approximately 1.0 to 1.2 mg/kg/min [10,115]. Brain glucose uptake is insulin-independent and accounts for approximately 50% to 60% of glucose disposal under fasting conditions. Therefore, brain glucose uptake occurs at the same rate during absorptive and postabsorptive periods, and it is not altered in type 2 diabetes. During glucose ingestion, insulin is secreted into the portal vein where it is taken up by the liver and suppresses hepatic glucose output. If the liver does not perceive this insulin signal and continues to produce glucose, there will be two inputs of glucose into the body, one from the liver and a second from the gastrointestinal tract, and marked hyperglycemia will ensue.

In type 2 diabetics with mild fasting hyperglycemia ($\leq$140 mg/dL), the postabsorptive level of hyperinsulinemia is sufficient to offset the hepatic insulin resistance and maintain a normal basal rate of hepatic glucose output [114]. In diabetic subjects with mild to moderate fasting hyperglycemia (140–200 mg/dL, 7.8–11.1 mmol/L), however, basal hepatic glucose production is increased by approximately 0.5 mg/kg/min (Fig. 7) [1,2,12,114]. Thus, during overnight sleeping hours (20:00 h to 08:00 h), the liver of an 80-kg diabetic individual with modest fasting hyperglycemia adds approximately 30 g of additional glucose to the systemic circulation. The increase in basal hepatic glucose production (HGP) is closely correlated with the severity of fasting hyperglycemia (see Fig. 7) [1,2,12,114], and this has been demonstrated in numerous studies [116–118]. In conclusion, in type 2 diabetics with overt fasting hyperglycemia ($\geq$140 mg/dL, 7.8 mmol/L), an excessive rate of hepatic glucose output is the major abnormality responsible for the elevated FPG concentration.

Under postabsorptive conditions, the fasting plasma insulin concentration in type 2 diabetics is 2- to 4-fold greater than in nondiabetic subjects [1,2]. Because hyperinsulinemia is a potent inhibitor of HGP, it is obvious that hepatic resistance to the action of insulin must be present to explain the excessive output of glucose by the liver. Because hyperglycemia per se exerts a powerful suppressive action on HGP, the liver also must be resistant to the inhibitory effect of hyperglycemia on hepatic glucose output, and this has been well documented.

The dose response relationship between hepatic glucose production and the plasma insulin concentration has been examined with the euglycemic

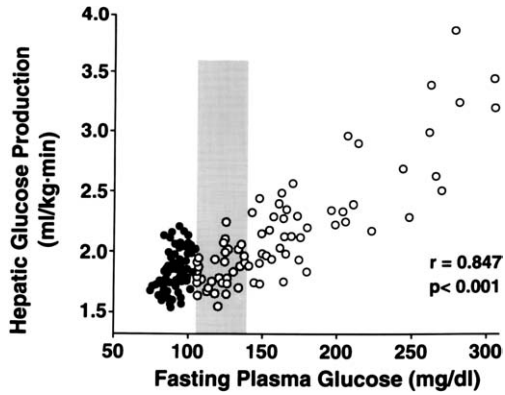

Fig. 7. Summary of hepatic glucose production (HGP) in 77 normal-weight type 2 diabetic subjects (○) with fasting plasma glucose concentrations ranging from 105 to greater than 300 mg/dL. Seventy-two control subjects matched for age and weight are shown by filled circles. In the 33 diabetic subjects with fasting plasma glucose levels less than 140 mg/dL (*shaded area*), the mean rate of hepatic glucose production was identical to that of control subjects. In diabetic subjects with fasting plasma glucose concentrations greater than 140 mg/dL, there was a progressive rise in hepatic glucose production that correlated closely (r, 0.847; $P \leq 0.001$) with the fasting plasma glucose concentration. (*From* DeFronzo RA, Ferrannini E, Simonson DC. Fasting hyperglycemia in non-insulin-dependent diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake. Metabolism 1989;38(4):387–95; with permission.)

insulin clamp technique and radioisotopic glucose (Fig. 8) [12]. The following points deserve emphasis: (1) the dose-response curve relating inhibition of HGP to the plasma insulin level is very steep, with a half-maximal insulin concentration (ED_{50}) of approximately 30 to 40 μU/mL; (2)

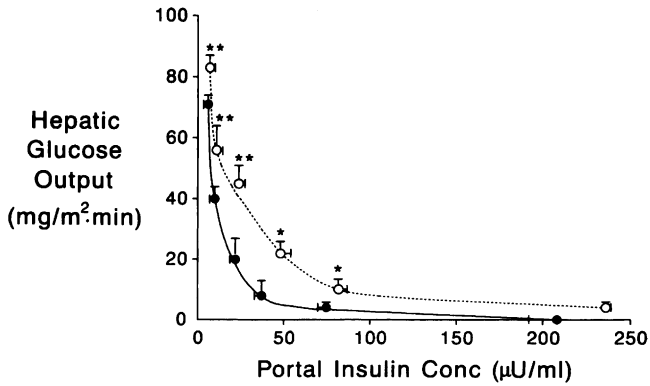

Fig. 8. Dose-response curve relating the plasma insulin concentration to the suppression of hepatic glucose production in control (●) and type 2 diabetic (○) subjects with moderately severe fasting hyperglycemia. * $P \leq 0.05$; ** $P \leq 0.01$ versus control subjects. (*From* Groop LC, Bonadonna RC, DelPrato S, Ratheiser K, Zyck K, Ferrannini E, DeFronzo RA. Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. J Clin Invest. 1989;84(1):205–13; with permission.)

in type 2 diabetics, the dose-response curve is shifted to the right, indicating resistance to the inhibitory effect of insulin on hepatic glucose production. Elevation of the plasma insulin concentration to the high physiologic range (~100 μU/mL), however, can overcome the hepatic insulin resistance and cause a near normal suppression of HGP; and (3) the severity of the hepatic insulin resistance is related to the level of glycemic control. In type 2 diabetics with mild fasting hyperglycemia, an increment in plasma insulin concentration of 100 μU/mL causes a complete suppression of HPG; however, in diabetic subjects with more severe fasting hyperglycemia, the ability of the same plasma insulin concentration to suppress HGP is impaired. These observations indicate that there is an acquired component of hepatic insulin resistance, which becomes progressively worse as the diabetic state decompensates over time.

Hepatic glucose production can be derived from either glycogenolysis or gluconeogenesis [9]. Using the hepatic vein catheter technique, the uptake by the liver of gluconeogenic precursors, especially lactate, has been shown to increased in type 2 diabetic subjects [119], and this has been confirmed with radioisotope turnover studies using radiolabeled lactate, alanine, glutamine, and glycerol [120,121]. More recent studies using ^{13}C-labeled magnetic resonance imaging [122] and D_2O [123] have confirmed that approximately 90% of the increase in HGP above baseline can be accounted for by accelerated gluconeogenesis. A variety of mechanisms has been shown to contribute to the increase in hepatic gluconeogenesis, including hyperglucagonemia, enhanced sensitivity to glucagon, increased circulating levels of gluconeogenic precursors (lactate, alanine, glycerol), increased FFA oxidation, and decreased sensitivity to insulin.

Because of the inaccessibility of the liver in humans, it has been difficult to examine the role of key enzymes involved in the regulation of gluconeogenesis (pyruvate carboxylase, phosphoenol-pyruvate carboxykinase), glycogenolysis (glycogen phosphorylase), and net hepatic glucose output (glucokinase, glucose-6-phosphatase). Considerable evidence from animal models of type 2 diabetes and some evidence in humans, however, has implicated increased activity of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase in the accelerated rate of hepatic glucose production [123,124].

The kidney possesses all of the gluconeogenesis enzymes required to produce glucose, and estimates of the renal contribution to total endogenous glucose production have ranged from 5% to 20% [125,126]. These varying estimates of the contribution of renal gluconeogenesis to total glucose production are largely related to the methodology used to measure renal glucose production [127]. One unconfirmed study suggests that the rate of renal gluconeogenesis is increased in type 2 diabetics with fasting hyperglycemia [128], but studies using the hepatic vein catheter technique have shown that all of the increase in total body endogenous glucose production (measured with [3-^{3}H]glucose) in type 2 diabetics can be accounted for by increased hepatic glucose output (measured by the hepatic vein catheter technique) [5].

Peripheral (muscle) glucose uptake

Muscle is the major site of insulin-stimulated glucose disposal in humans [1–3,5,129,130]. Under euglycemic conditions, studies using the euglycemic insulin clamp in combination with femoral artery or vein catheterization have shown that approximately 80% of total body glucose uptake occurs in skeletal muscle. In response to a physiologic increase in plasma insulin concentration (~80–100 μU/mL), leg muscle glucose uptake increases progressively in healthy subjects and reaches a plateau value of approximately 10 mg/kg leg weight/min (Fig. 9) [5]. In contrast, in lean type 2 diabetic subjects, the onset of insulin action is delayed by approximately 40 min, and the amount of glucose taken up by the leg is markedly blunted, even though the insulin infusion is continued for an additional 60 min to allow insulin to more fully express its biologic effects. During the last hour of the insulin clamp study, the rate of glucose uptake was reduced by 50% in the type 2 diabetic group. These results provide conclusive evidence that muscle represents the primary site of insulin resistance during euglycemic insulin clamp studies performed in type 2 diabetic subjects. Using the forearm and leg catheterization techniques, a number of investigators have demonstrated a decrease in insulin-stimulated muscle glucose uptake. Studies using positron emission tomography have provided additional support for the presence of severe muscle insulin resistance in type 2 diabetic subjects.

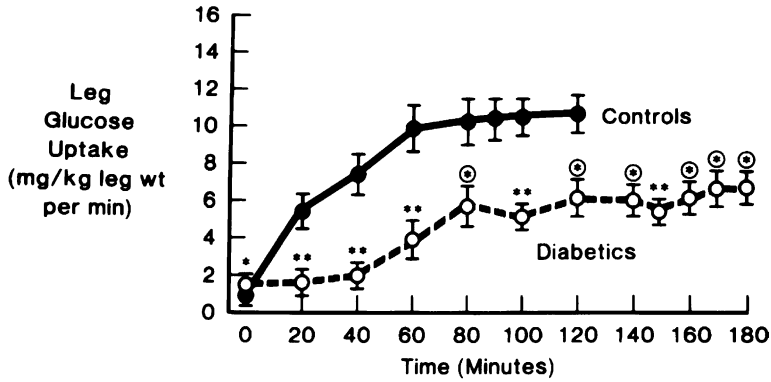

Fig. 9. Time course of change in leg glucose uptake in type 2 diabetic (○) and control (●) subjects. In the postabsorptive state, glucose uptake in the diabetic group was significantly greater than that in control subjects. The ability of insulin (euglycemic insulin clamp) to stimulate leg glucose uptake, however, was reduced by 50% in the diabetic subjects. * $P < 0.05$; ** $P < 0.01$. (*From* DeFronzo RA, Gunnarsson R, Bjorkman O, Olsson M, Wahren J. Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. J Clin Invest 1985;76(1):149–55; with permission.)

Splanchnic (hepatic) glucose uptake

Because of the difficulty in catheterizing the portal vein in humans, glucose disposal by the liver has not been examined directly. Use of the hepatic vein catheterization technique in combination with the euglycemic insulin clamp, however, has allowed investigators to examine the contribution of the splanchnic (liver plus gastrointestinal) tissues to overall glucose homeostasis in lean type 2 diabetic subjects [3,5,129]. In the postabsorptive state, there is a net release of glucose from the splanchnic area (ie, negative balance) in both control and diabetic subjects, reflecting glucose production by the liver. When insulin is infused while maintaining euglycemia, splanchnic glucose output is promptly suppressed (reflecting inhibition of HGP) and, within 20 min, the net glucose balance across the splanchnic region decreases to zero (i.e., no net uptake or release). After 2 hours of sustained hyperinsulinemia, the splanchnic area manifests a small net uptake of glucose, approximately 0.5 mg/kg/min (i.e., positive balance), which is virtually identical to the rate of splanchnic glucose uptake during in the basal state. These results indicate that the splanchnic tissues, like the brain, are largely insensitive to insulin with respect to the stimulation of glucose uptake. There were no differences between diabetic and control subjects in the amount of glucose taken up by the splanchnic tissues at any time during the insulin clamp study.

In summary, under conditions of euglycemic hyperinsulinemia, very little infused glucose is taken up by the splanchnic (and therefore hepatic) tissues. Because the difference in insulin-mediated total body glucose uptake between type 2 diabetic and control subjects during the euglycemic insulin clamp study was 2.5 mg/kg/min, it is obvious that a defect in splanchnic (hepatic) glucose removal cannot account for the impairment in total body glucose uptake following intravenous glucose/insulin administration; however, following glucose ingestion, the gastrointestinal route of glucose entry and the resultant hyperglycemia conspire to enhance splanchnic (hepatic) glucose uptake [4,9,16,17,131] and, under these conditions, diminished hepatic glucose uptake has been shown to contribute to impaired glucose tolerance in type 2 diabetes (see discussion below).

Summary: whole-body glucose use

A summary of insulin-mediated whole-body glucose metabolism during the euglycemic insulin clamp is depicted in (Fig. 10). The height of each bar represents the total amount of glucose taken up by all tissues in the body during the insulin clamp in control and type 2 diabetic subjects. Net splanchnic glucose uptake (quantitated by hepatic vein catheterization) is similar in both groups and averaged 0.5 mg/kg/min. Adipose tissue glucose uptake accounts for no more than 5% of total glucose disposal. Brain glucose uptake, estimated to be 1.0–1.2 mg/kg/min in the postabsorptive state, is unaffected by hyperinsulinemia. Muscle glucose uptake (extrapolated from leg catheterization data) in control subjects accounts for

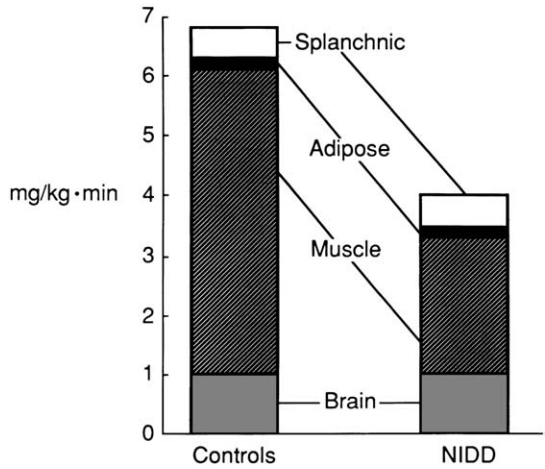

Fig. 10. Summary of glucose metabolism during euglycemic insulin (100 μU/mL) clamp studies performed in normal-weight type 2 diabetic and control subjects (see text for a more detailed discussion). NIDD, non-insulin-dependent diabetes. (*From* DeFronzo RA. Pathogenesis of type 2 diabetes mellitus: metabolic and molecular implications for identifying diabetes genes. Diabetes 1997;5:117–269; with permission.)

approximately 75%–80% of glucose uptake by the body. In type 2 diabetic subjects, the largest part of the impairment in insulin-mediated glucose uptake is explained by the defect in muscle glucose disposal. Numerous studies have demonstrated that adipocytes from type 2 diabetics are resistant to insulin, but because the total amount of glucose taken up by fat cells during the insulin clamp is small [130], even if adipose tissue of type 2 diabetic subjects took up no glucose, it could, at best, explain only a small fraction of the defect in whole-body glucose metabolism.

Glucose disposal during OGTT

During daily life, the normal route of glucose entry into the body is through the gastrointestinal tract. To assess tissue glucose disposal following glucose ingestion, Ferrannini, DeFronzo, and colleagues [131–133] administered oral glucose combined with hepatic vein catheterization to healthy control subjects to examine splanchnic glucose metabolism. The oral glucose load and endogenous glucose pool were labeled with [1-^{14}C]glucose and [3-^{3}H]glucose, respectively, to quantitate total body glucose disposal (from tritiated glucose turnover) and endogenous HGP (difference between the total rate of glucose appearance, measured with tritiated glucose, and the rate of oral glucose appearance, measured with [1-^{14}C]glucose).

During the 3.5 hours after glucose (68 g) ingestion: (1) 28% (19 g) of the oral load was taken up by splanchnic tissues; (2) 72% (48 g) was removed by nonsplanchnic tissues; (3) of the 48 g taken up by peripheral tissues, the

brain (an insulin-independent tissue) disposed of 22% (15 g or 1 mg/kg/min) of the total glucose load; and (4) basal HGP declined by 53% [131]. Similar percentages for splanchnic glucose uptake (24%–29%) and suppression of HGP (50%–60%) in normal subjects have been reported by other investigators [8,134–136]. The contribution of skeletal muscle to the disposal of an oral glucose load has been reported to vary from a low of 26% [135] to a high of 56% [136], with a mean of 45% [8,131,134–136]. These results demonstrate a number of important differences between oral and intravenous glucose administration. After glucose ingestion, HGP is less completely suppressed, most likely because of activation of local sympathetic nerves that innervate the liver, peripheral tissue (primarily muscle) glucose uptake is quantitatively less important [3], and splanchnic glucose uptake quantitatively is much more important.

When an oral glucose is given to type 2 diabetic individuals, marked glucose intolerance is observed, and this results from decreased tissue (muscle) glucose uptake and impaired suppression of HGP. The uptake of glucose by the splanchnic tissues is similar in diabetic and control groups. Impaired suppression of HGP accounts for approximately one third of the defect in total-body glucose homeostasis, whereas reduced peripheral (muscle) glucose uptake accounts for the remaining two thirds. It should be noted that, even though the total amount of glucose taken up by the splanchnic region in type 2 diabetics is ''normal,'' splanchnic glucose metabolism is quite abnormal. Because hyperglycemia per se enhances splanchnic (hepatic) glucose uptake in proportion to the increase in plasma glucose concentration, and because the rise in plasma glucose concentration in diabetics is excessive, the splanchnic glucose clearance (splanchnic glucose uptake ÷ plasma glucose concentration) following glucose ingestion is markedly reduced in type 2 diabetic subjects. Using a combined insulin clamp/OGTT technique, an impairment in glucose uptake by the splanchnic tissues in type 2 diabetics has been demonstrated directly [137].

In summary, following glucose ingestion both impaired suppression of HGP and decreased muscle glucose uptake are responsible for the glucose intolerance of type 2 diabetes. The efficiency of the splanchnic (hepatic) tissues to take up glucose (as reflected by the splanchnic glucose clearance) also is impaired in type 2 diabetic individuals.

Summary: insulin resistance in type 2 diabetes

Insulin resistance in muscle and liver is a characteristic feature of type 2 diabetes mellitus. In the basal state, the hepatic insulin resistance is manifested by overproduction of glucose despite fasting hyperinsulinemia, and the increased rate of hepatic glucose output is the primary determinant of the elevated FPG concentration in type 2 diabetic individuals. Although muscle glucose uptake in the postabsorptive state is increased when viewed in absolute terms, the efficiency with which glucose is taken up (ie, the glucose

clearance) by muscle is diminished. During insulin-stimulated conditions, both decreased muscle glucose uptake and impaired suppression of HGP contribute to the glucose intolerance.

Dynamic interaction between insulin sensitivity and insulin secretion in type 2 diabetes

Insulin resistance is present in approximately 25% of the adult population [138–140]. The majority of these individuals, however, have normal glucose tolerance because the pancreatic beta cells are able to read the severity of insulin resistance and appropriately augment their insulin secretory rate. This dynamic interaction between insulin sensitivity and insulin secretion is demonstrated by results obtained in healthy, lean, young normal-glucose-tolerant women who received a euglycemic insulin clamp (1 mU/kg/min) and were stratified into quartiles based on the rate of insulin-mediated glucose disposal (see Fig. 2A) [141]. Women in the lowest quartile were as insulin resistant as type 2 diabetic individuals. Insulin secretion was measured on a separate day with a +125-mg/dL hyperglycemic clamp (see Fig. 2B). Women who were the most insulin resistant (quartile 1) had the highest fasting plasma insulin concentrations and highest early and late-phase plasma insulin responses (see Fig. 2B). Conversely, women who were the most insulin sensitive (quartile 4) had the lowest plasma insulin response. A very strong positive correlation (r, 0.79, $P \leq 0.001$) was observed between the severity of insulin resistance and the insulin secretory response. Similar results relating the plasma insulin response and the severity of insulin resistance have been reported in normal-glucose-tolerant subjects with the minimal model technique and the insulin suppression/OGTT.

The dynamic interaction between insulin secretion and insulin sensitivity in type 2 diabetic individuals has been the subject of intensive investigation. DeFronzo [2] studied lean (ideal body weight $\leq$ 120%) and obese (ideal body weight $\geq$ 125%) subjects with varying degrees of glucose tolerance: group I, obese subjects (n = 24) with normal glucose tolerance; group II, obese subjects (n = 23) with impaired glucose tolerance; group III, obese subjects (n = 35) with overt diabetes, who were subdivided into those with a hyperinsulinemic response and those with a hypoinsulinemic response during an OGTT; group IV, normal weight type 2 diabetics (n = 26); and group V, normal weight subjects (n = 25) with normal glucose tolerance (see Fig. 4). All subjects received a euglycemic insulin ($\sim$100 μU/mL) clamp to quantitate whole-body insulin sensitivity and an OGTT to provide a measure of glucose tolerance and insulin secretion. The insulin clamp was performed with indirect calorimetry to quantitate rates of glucose oxidation and nonoxidative glucose disposal, which primarily reflects glycogen synthesis.

In normal weight type 2 diabetic subjects, insulin-mediated whole-body glucose uptake was reduced by 40%–50%, and the impairment in insulin

action resulted from defects in both glucose oxidation and glycogen synthesis. It is particularly noteworthy that obese, normal glucose tolerant individuals were as insulin resistant as the lean normal-weight diabetic subjects (see Fig. 4) and that the insulin resistance resulted from defects in both glucose oxidation and glycogen synthesis. Thus, from the metabolic standpoint, obesity and type 2 diabetes closely resemble each other. Similar results concerning reduced whole-body insulin sensitivity in obese and type 2 diabetic individuals have been reported by other investigators [142,143]. Despite nearly identical degrees of insulin resistance, the normal-weight diabetic subjects (see Fig. 4) had fasting hyperglycemia and marked glucose intolerance, whereas the obese nondiabetic individuals had normal oral glucose tolerance. This apparent paradox is explained by the plasma insulin response during the OGTT (see Fig. 4). Compared with control subjects, the obese group secreted more than twice as much insulin, and this hyperinsulinemic response was sufficient to offset the insulin resistance. In contrast, the pancreas of normal-weight diabetic subjects, when faced with the same challenge, was unable to augment its secretion of insulin sufficiently to compensate for the insulin resistance. This imbalance between insulin secretion and the severity of insulin resistance in liver and muscle resulted in a frankly diabetic state, with fasting hyperglycemia and marked glucose intolerance.

The coexistence of obesity and diabetes in the same individual resulted in a severity of insulin resistance that was only slightly greater than that in either the normal-weight diabetic or nondiabetic obese groups (see Fig. 4). Although hyperinsulinemic and hypoinuslinemic obese diabetic subjects were equally insulin resistant, the glucose intolerance was much worse in the hypoinsulinemic group, and this was related entirely to the presence of severe insulin deficiency (see Fig. 4).

The interaction between insulin secretion and insulin resistance in lean, obese, and diabetic groups can be summarized as follows. In the obese nondiabetic subjects, tissue sensitivity to insulin (Fig. 4, top) is markedly reduced, but glucose tolerance (bottom) remains normal because the pancreas is able to augment its secretion of insulin (top) to offset the defect in insulin action. The development of IGT, the mean plasma glucose concentration during the OGTT, increases only minimally because the pancreas is able to further augment its secretion of insulin to counteract the deterioration in insulin sensitivity. Progression from IGT to overt diabetes is signaled by a decrease in insulin secretion without any worsening of insulin resistance (see Fig. 4). The obese diabetic has tipped over the top of Starling's curve of the pancreas and is now on the descending portion (see Figs. 3 and 4). Although, compared with nondiabetic control subjects, the plasma insulin response is increased, the hyperinsulinemia is insufficient to offset the severity of insulin resistance. In the normal-weight diabetic group, there is a further decline in glucose tolerance, which results from a greater impairment in insulin secretion without any additional deterioration in

insulin sensitivity. Last, the obese diabetic group with a low insulin response manifests the greatest glucose intolerance owing to the presence of marked insulin deficiency without further worsening of insulin sensitivity (see Fig. 4).

The natural history of type 2 diabetes described above (see Fig. 4) is consistent with that described by other investigators in humans and monkeys [1,2,20,22–27,29,33–35,97–99,102,144–146]. In lean subjects spanning a wide range of glucose tolerance, Reaven et al [97] demonstrated that the progression from normal glucose tolerance to IGT was signaled by the development of severe insulin resistance, which was largely counterbalanced by increased insulin secretion. Progression from IGT to type 2 diabetes was associated with a marked decline in insulin secretion with no (or only slight) further deterioration in tissue sensitivity to insulin (Fig. 11). A similar sequence of events has been documented prospectively in Pima Indians, Pacific Islanders, and rhesus monkeys.

In summary, insulin resistance is an early and characteristic feature of the natural history of type 2 diabetes in high-risk populations. Overt diabetes develops only when the beta cells are unable to appropriately augment their secretion of insulin to compensate for the defect in insulin action. It should be recognized, however, that there are well-described type 2 diabetic populations in whom insulin sensitivity is normal at the onset of diabetes, whereas insulin secretion is severely impaired. This insulinopenic variety of type 2 diabetes appears to be more common in African Americans, elderly subjects, and lean whites. In this latter group, it is important to exclude type 1 diabetes, because approximately 10% of white individuals with older onset diabetes are islet cell antibody, or glutamic acid decarboxylase, positive.

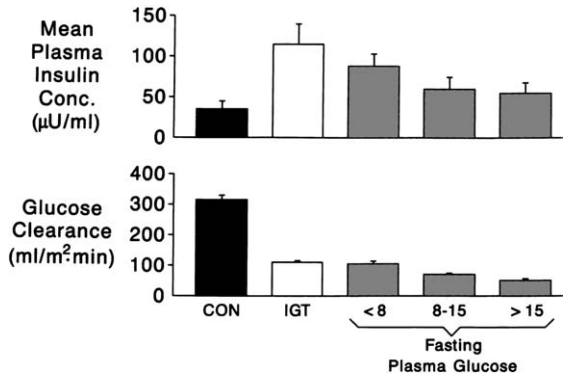

Fig. 11. Insulin-mediated glucose clearance (measured with the insulin suppression test) and the plasma insulin response (measured with an OGTT) in controls (*top*), in subjects with IGT (*bottom*), and in type 2 diabetic individuals (*top*) with varying severity of glucose intolerance (see text for a more detailed discussion). (*Data from* Reaven GM, Hollenbeck CB, Chen YDI. Relationship between glucose tolerance, insulin secretion, and insulin action in non-obese individuals with varying degrees of glucose tolerance. Diabetologia 1989;32:52–5.)

Role of the adipocyte in the pathogenesis of type 2 diabetes mellitus: the harmonious quartet

The majority ($\geq$ 80%–90%) of type 2 diabetics in the United States are overweight or obese [147]. Both lean and especially obese type 2 diabetics are characterized by day-long elevation in plasma free fatty-acid concentration, which fails to suppress normally following ingestion of a mixed meal or oral glucose load [30]. FFA are stored as triglycerides in adipocytes and serve as an essential energy source during fasting conditions. Insulin is a potent antilipolytic hormone and restrains the release of FFA from the adipocyte by inhibiting the enzyme hormone-sensitive lipase [11,12]. The fat cells of type 2 diabetics (and nondiabetic obese individuals) are markedly resistant to the inhibitory effect of insulin on lipolysis. In the postabsorptive state, the rate of lipolysis (as reflected by impaired suppression of radioactive palmitate turnover) is increased despite plasma insulin levels that are 2- to 4-fold elevated. Moreover, the ability of exogenous insulin to inhibit the elevated basal rate of lipolysis and to reduce the plasma FFA concentration is markedly impaired. Many studies have shown that chronically elevated plasma FFA concentrations cause insulin resistance in muscle and liver and impair insulin secretion (Fig. 12) [1,2,11,14,58,148–152]. Thus, increased plasma FFA levels can cause or aggravate the three major pathogenic disturbances that are responsible for impaired glucose homeostasis in type 2 diabetic individuals, and the time has arrived for the "triumvirate" (muscle, liver, beta cell) to be joined by the "fourth musketeer" [152] to form the "harmonious quartet" (Fig. 13) [11]. In addition to the FFA that circulate in plasma in increased amounts, type 2 diabetic and obese nondiabetic individuals have increased stores of triglycerides in muscle and liver, and the increased fat content correlates closely with the presence of insulin resistance in these tissues [153–156]. Triglycerides in liver and muscle are in a state of constant turnover, and the intracellular metabolites of triglycerides and FFA (ie, fatty acyl-CoA, ceramides, and diacylglycerol) have been shown to impair insulin action

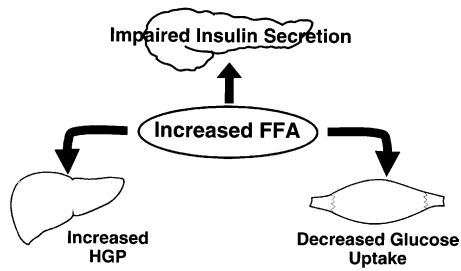

Fig. 12. Etiology of type 2 diabetes mellitus (T2DM). The deleterious effect of chronically elevated plasma FFA concentrations on basal or insulin-suppressed rate of hepatic glucose production, insulin-stimulated glucose uptake in muscle, and glucose-stimulated insulin secretion.

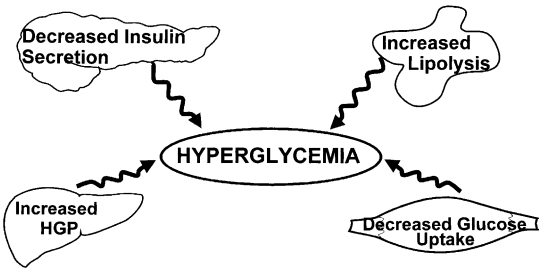

Fig. 13. Harmonious quartet. Insulin resistance in adipocytes, muscle, and liver in combination with impaired insulin secretion by the pancreatic beta cells, represent the four major organ system abnormalities that play a central role in the pathogenesis of type 2 diabetes mellitus.

in both liver and muscle [11,157–159]. Evidence also has accumulated to implicate lipotoxicity as an important cause of beta-cell dysfunction (see earlier discussion) [11,58,150,151]. The sequence of events whereby elevated plasma FFA and increased lipid deposition in tissues cause insulin resistance and promote beta-cell failure has been referred to as "lipotoxicity," and several recent in depth reviews of this subject have been published [11,58].

Cellular mechanisms of insulin resistance

The cellular events through which insulin initiates its stimulatory effect on glucose metabolism start with binding of the hormone to specific receptors that are present on the cell surface of all insulin target tissues [2,160–162]. After insulin has bound to and activated its receptor, "second messengers" are generated, and these second messengers activate a cascade of phosphorylation-dephosphorylation reactions that eventually result in the stimulation of intracellular glucose metabolism. The first step in glucose use involves activation of the glucose transport system, leading to glucose influx into insulin target tissues, primarily muscle. The free glucose, which has entered the cell, subsequently is metabolized by a series of enzymatic steps that are under the control of insulin. Of these, the most important are glucose phosphorylation (catalyzed by hexokinase), glycogen synthase (which controls glycogen synthesis), and phosphofructokinase (PFK) and pyruvate dehydrogenase (PDH) (which regulate glycolysis and glucose oxidation, respectively).

Insulin receptor/insulin receptor tyrosine kinase

The insulin receptor is a glycoprotein that consists of two α-subunits and two β-subunits linked by disulfide bonds (Fig. 14) [2,160–162]. The two α-subunits of the insulin receptor are entirely extracellular and contain the insulin-binding domain. The β-subunits have an extracellular domain, a transmembrane domain, and an intracellular domain that expresses

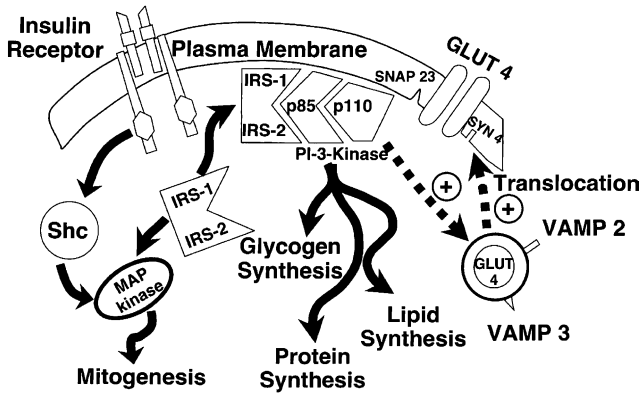

Fig. 14. Insulin transduction system. Insulin receptor and the cascade of intracellular signaling molecules that have been implicated in insulin action (see text for a more detailed discussion).

insulin-stimulated kinase activity directed toward its own tyrosine residues. Phosphorylation of the β-subunit, with subsequent activation of insulin receptor tyrosine kinase, represents the first step in the action of insulin on glucose metabolism. Mutagenesis of any of the three major phosphorylation sites (at residues 1158, 1163, and 1162) impairs insulin receptor kinase activity, leading to a decrease in the metabolic and growth-promoting effects of insulin [163,164].

Insulin receptor signal transduction

Following its activation, insulin receptor tyrosine kinase phosphorylates specific intracellular proteins, of which at least nine have been identified [160,165]. In muscle insulin-receptor substrate (IRS)-1 serves as the major docking protein that interacts with the insulin receptor tyrosine kinase and undergoes tyrosine phosphorylation in regions containing specific amino acid sequence motifs that, when phosphorylated, serve as recognition sites for proteins containing *src*-homology 2 (SH2) domains. Mutation of these specific tyrosines severely impairs the ability of insulin to stimulate muscle glycogen synthesis, glucose oxidation, and other acute metabolic- and growth-promoting effects of insulin [164]. In liver, IRS-2 serves as the primary docking protein that undergoes tyrosine phosphorylation and mediates the effect of insulin on hepatic glucose production, gluconeogenesis, and glycogen formation [166].

In muscle, the phosphorylated tyrosine residues of IRS-1 mediate an association with the 85-kDa regulatory subunit of phosphatidylinositol 3-kinase (PI3K), leading to activation of the enzyme (see Fig. 14) [160–162,165,167]. PI3K is composed of an 85-kDa regulatory subunit and a 110-kDa catalytic subunit. The latter catalyzes the 3′ phosphorylation of PI 4-phosphate and PI 4,5-diphosphate, resulting in the stimulation of glucose

transport. Activation of PI3K by phosphorylated IRS-1 also leads to activation of glycogen synthase through a process that involves activation of protein kinase B/Akt and subsequent inhibition of kinases, such as glycogen synthase kinase-3, and activation of protein phosphatase 1 (PP1) (also called glycogen synthase phosphatase, see later discussion). Inhibitors of PI3K impair glucose transport and block the activation of glycogen synthase and hexokinase (HK)-II expression [160–162,165,167–169]. The action of insulin to increase protein synthesis and inhibit protein degradation also is mediated by PI3K.

Other proteins with SH2 domains, including the adapter protein Grb2 and *Shc*, also interact with IRS-1 and become phosphorylated following exposure to insulin [160–162,165]. Grb2 and *Shc* link IRS-1/IRS-2 to the mitogen-activated protein kinase (MAPK)-signaling pathway (see Fig. 14), which plays an important role in the generation of transcription factors and promotes cell growth, proliferation, and differentiation [160,165]. Inhibition of the MAPK kinase pathway prevents the stimulation of cell growth by insulin but has no effect on the metabolic actions of the hormone [170].

Under anabolic conditions, insulin augments glycogen synthesis by simultaneously activating glycogen synthase and inhibiting glycogen phosphorylase [171,172]. The effect of insulin is mediated through the PI3K pathway, which inactivates kinases such as glycogen synthase kinase-3 and activates phosphatases, particularly PP1. PP1 is believed to be the primary regulator of glycogen metabolism. In skeletal muscle, PP1 associates with a specific glycogen-binding regulatory subunit, causing dephosphorylation (activation) of glycogen synthase. PP1 also phosphorylates (inactivates) glycogen phosphorylase. The precise steps that link insulin receptor tyrosine kinase/PI3K activation to stimulation of PP1 have yet to be defined. Studies [160,173] have demonstrated convincingly that inhibitors of PI3K inhibit glycogen synthase activity and abolish glycogen synthesis.

Insulin receptor signal transduction defects in type 2 diabetes

Insulin receptor number and affinity

Both receptor and postreceptor defects have been shown to contribute to insulin resistance in individuals with type 2 diabetes mellitus. Some studies have demonstrated a modest 20%–30% reduction in insulin binding to monocytes and adipocytes from type 2 diabetic patients, but this has not been a consistent finding [1,174–177]. The decrease in insulin binding is caused by a reduction in the number of insulin receptors without change in insulin receptor affinity. Some caution, however, should be used in interpreting these studies because muscle and liver not adipocytes are the major tissues responsible for the regulation of glucose homeostasis in vivo, and insulin binding to solubilized receptors obtained from skeletal muscle and liver has been shown to be normal in obese and lean diabetic individuals [175,176,178]. Moreover, a decrease in insulin receptor number cannot be

demonstrated in over half of type 2 diabetic subjects, and it has been difficult to demonstrate a correlation between reduced insulin binding and the severity of insulin resistance [179–181]. A variety of defects in insulin receptor internalization and processing have been described in syndromes of severe insulin resistance and diabetes. The insulin receptor gene, however, has been sequenced in a large number of type 2 diabetic patients from diverse ethnic populations and, with very rare exceptions, physiologically significant mutations in the insulin receptor gene have not been observed [182,183]. This excludes a structural gene abnormality in the insulin receptor as a cause of common type 2 diabetes mellitus.

Insulin receptor tyrosine kinase activity

Insulin receptor tyrosine kinase activity has been examined in skeletal muscle, adipocytes, and hepatocytes from normal-weight and obese diabetic subjects. Most [1,175,176,179,184,185] but not all [178] investigators have found a reduction in tyrosine kinase activity (Fig. 15) that cannot be explained by alterations in insulin receptor number or insulin receptor binding affinity. Restoration of normoglycemia by weight loss, however, has been shown to correct the defect in insulin receptor tyrosine kinase activity [186], suggesting that the defect in tyrosine kinase is acquired secondary to some combination of hyperglycemia, distributed intracellular glucose metabolism, hyperinsulinemia, and insulin resistance, all of which improved after weight loss. Exposure of cultured fibroblasts to high glucose concentration also has been shown to

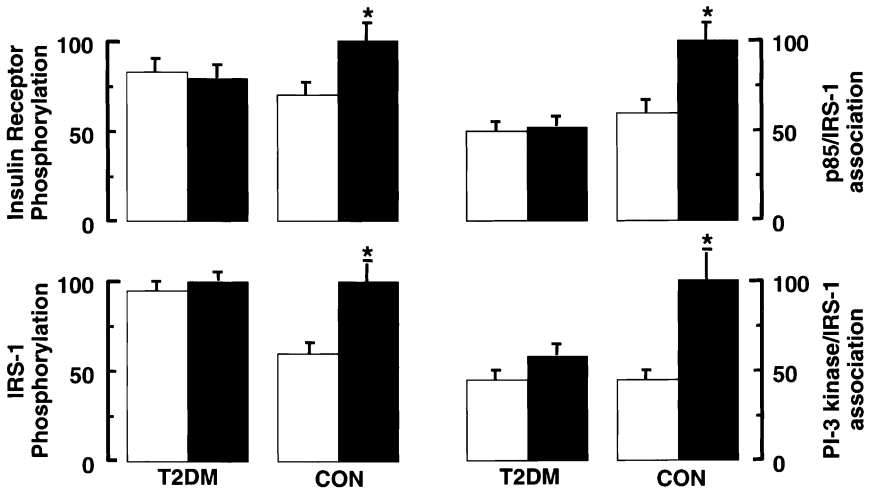

Fig. 15. Insulin signaling cascade in T2DM. Effect of insulin on insulin receptor (*top*) and IRS-1 tyrosine phosphorylation (*bottom*) and the association of IRS-1 with the p85 regulatory subunit of PI3K and PI3K activity in muscle from T2DM and control (CON) subjects. Data are expressed as percentages of the mean insulin-stimulated values in the control groups. Open bars, basal state; filled bars, insulin-stimulated state; * $P \leq 0.05$, T2DM versus CON.

inhibit insulin receptor tyrosine kinase activity [187]. Because insulin receptor tyrosine kinase activity assays are performed in vitro, the results of these assays could provide misleading information with regard to insulin receptor function in vivo. To circumvent this problem, investigators have used the euglycemic hyperinsulinemic clamp with muscle biopsies and anti-phosphotyrosine immunoblot analysis to provide a ''snap shot'' of the insulin-stimulated tyrosine phosphorylation state of the receptor in vivo [185]. In insulin-resistant obese nondiabetic and type 2 diabetic subjects, a substantial decrease in insulin receptor tyrosine phosphorylation has been demonstrated; however, when insulin-stimulated insulin receptor tyrosine phosphorylation was examined in normal-glucose-tolerant insulin-resistant individuals (offspring of two diabetic parents) at high risk for developing type 2 diabetes, a normal increase in tyrosine phosphorylation of the insulin receptor was observed [188]. These findings are consistent with the concept that impaired insulin receptor tyrosine kinase activity in type 2 diabetic patients is acquired secondary to hyperglycemia or some other metabolic disturbance.

Insulin-signaling (IRS-1 and PI3K) defects

In insulin-resistant obese nondiabetic subjects, the ability of insulin to activate insulin receptor and IRS-1 tyrosine phosphorylation in muscle is modestly reduced, whereas in type 2 diabetics insulin-stimulated insulin receptor and IRS-1 tyrosine phosphorylation are severely impaired (see Fig. 15) [185]. Association of the p85 subunit of PI3K with IRS-1 and activation of PI3K also are greatly attenuated in obese nondiabetic and type 2 diabetic subjects compared with lean healthy controls (see Fig. 15) [185,189,190]. The decrease in insulin-stimulated association of the p85 regulatory subunit of PI3K with IRS-1 is closely correlated with the reduction in insulin-stimulated muscle glycogen synthase activity and in vivo insulin-stimulated glucose disposal [185]. Impaired regulation of PI3K gene expression by insulin also has been demonstrated in skeletal muscle and adipose tissue of type 2 diabetic subjects [191]. In animal models of diabetes, an 80%–90% decrease in insulin-stimulated IRS-1 phosphorylation and PI3K activity has been reported [192].

In the insulin-resistant, normal glucose tolerant offspring of two type 2 diabetic parents, IRS-1 tyrosine phosphorylation and the association of p85 protein/PI3K activity with IRS-1 are markedly decreased despite normal tyrosine phosphorylation of the insulin receptor; these insulin signaling defects are correlated closely with the severity of insulin resistance measured with the euglycemic insulin clamp technique [188]. In summary, impaired association of PI3K with IRS-1 and its subsequent activation are characteristic abnormalities in type 2 diabetics, and these defects are correlated closely with in vivo muscle insulin resistance. A common mutation in the IRS-1 gene (Gly-972-Arg) has been associated with type 2 diabetes, insulin resistance, and obesity, but the physiologic significance of this mutation remains to be established [193].

Insulin resistance of the PI3K signaling pathway contrasts with an intact stimulation of the MAPK pathway by insulin in insulin-resistant type 2 diabetic and obese nondiabetics individuals [185,189]. Physiologic hyperinsulinemia increases mitogen-activated protein kinase/extracellular signal-regulated kinase 1 activity (MEK-1) and extracellular signal-regulated kinase1/2 phosphorylation activity (ERK) similarly in lean healthy subjects, insulin-resistant obese nondiabetic, and type 2 diabetic patients. Intact stimulation of the MAPK pathway by insulin in the presence of insulin resistance in the PI3K pathway may play an important role in the development of atherosclerosis [185]. If the metabolic (PI3K) pathway is impaired, plasma glucose levels rise, resulting in increased insulin secretion and hyperinsulinemia. Because insulin receptor function is normal or only modestly impaired, especially early in the natural history of type 2 diabetes, this leads to excessive stimulation of the MAPK (mitogenic) pathway in vascular tissues, with resultant proliferation of vascular smooth muscle cells, increased collagen formation, and increased production of growth factors and inflammatory cytokines [194,195].

Glucose transport

Activation of the insulin signal transduction system in insulin target tissues stimulates glucose transport through a mechanism that involves translocation of a large intracellular pool of glucose transporters (associated with low-density microsomes) to the plasma membrane and their subsequent activation after insertion into the cell membrane [196,197]. There are five major, different facilitative glucose transporters (GLUT) with distinctive tissue distributions (Table 1) [198,199]. GLUT4, the insulin regulatable transporter, is found in insulin-sensitive tissues (muscle and adipocytes), has a K_m of approximately 5 mmol/L, which is close to that of the plasma glucose concentration, and is associated with HK-II [198,199]. In adipocytes and muscle, GLUT4 concentration in the plasma membrane increases markedly after exposure to insulin, and this increase is associated with

Table 1
Classification of glucose transport and HK activity according to their tissue distribution and functional regulation

Organ	Glucose transporter	Hexokinase computer	Classification
Brain	GLUT1	HK-I	Glucose dependent
Erythrocyte	GLUT1	HK-I	Glucose dependent
Adipocyte	GLUT4	HK-II	Insulin dependent
Muscle	GLUT4	HK-II	Insulin dependent
Liver	GLUT2	HK-IVL	Glucose sensor
Glucokinase beta cell	GLUT2	HK-IVB (glucokinase)	Glucose sensor
Gut	GLUT3-symporter	—	Sodium dependent
Kidney	GLUT3-symporter	—	Sodium dependent

Data from DeFronzo RA. Pathogenesis of type 2 diabetes mellitus: metablic and molecular implications for identifying diabetes genes. Diabetes 1997;5:177–269.

a reciprocal decline in the intracellular GLUT4 pool. GLUT1 is the predominant glucose transporter in the insulin-independent tissues (brain and erythrocytes) but also is found in muscle and adipocytes. GLUT1 is located primarily in the plasma membrane where its concentration is unchanged following exposure to insulin. It has a low K_m (~1 mmol/L) and is well suited for its function, which is to mediate basal glucose uptake. It is found in association with HK-I [200]. GLUT2 is the predominant transporter in liver and pancreatic beta cells where it is found in association with a specific hexokinase, HK-IV or glucokinase [201]. GLUT2 has a very high K_m (~15–20 mmol/L), which allows the glucose concentration in cells expressing this transporter to increase in direct proportion to the increase in plasma glucose concentration. This unique characteristic allows these cells to function as glucose sensors.

In adipocytes and muscle of type 2 diabetic patients, glucose transport activity is severely impaired [179,196,197,202–204]. In adipocytes from human and rodent models of type 2 diabetes, GLUT4 mRNA and protein content are markedly reduced, and the ability of insulin to elicit a normal translocation response and to activate the GLUT4 transporter after insertion into the cell membrane is decreased. In contrast to the adipocytes, muscle tissue from lean and obese type 2 diabetic subjects exhibits normal or increased levels of GLUT4 mRNA expression and normal levels of GLUT4 protein, thus demonstrating that transcriptional and translational regulation of GLUT4 is not impaired [205,206]. In contrast to the normal expression of GLUT4 protein and mRNA in muscle of type 2 diabetic subjects, every study that has examined adipose tissue has reported reduced basal and insulin-stimulated GLUT4 mRNA levels and decreased GLUT4 transporter number in all subcellular fractions. These observations demonstrate that GLUT4 expression in humans is subject to tissue-specific regulation. Using a novel triple-tracer technique, the in vivo dose-response curve for the action of insulin on glucose transport in forearm skeletal muscle has been examined in type 2 diabetic subjects, and insulin-stimulated inward muscle glucose transport has been shown to be severely impaired [207,208]. Impaired in vivo muscle glucose transport in type 2 diabetics also has been demonstrated using magnetic resonance imaging [209] and positron emission tomography [210]. Because the number of GLUT4 transporters in the muscle of diabetic subjects is normal, impaired GLUT4 translocation and decreased intrinsic activity of the glucose transporter must be responsible for the defect in muscle glucose transport. Large populations of type 2 diabetics have been screened for mutations in the GLUT4 gene [211]. Such mutations are very uncommon and, when detected, have been of questionable physiologic significance.

Glucose phosphorylation

Glucose phosphorylation and glucose transport are tightly coupled phenomena [212]. Hexokinase isoenzymes (HK-I–IV) catalyze the first

committed step of glucose metabolism, the intracellular conversion of free glucose to glucose-6-phosphate (Glu-6-P) (see Table 1) [198–200,213]. HK-I, HK-II, and HK-III are single-chain peptides that have a very high affinity for glucose and demonstrate product inhibition by Glu-6-P. HK-IV, also called glucokinase, has a lower affinity for glucose and is not inhibited by Glu-6-P. Glucokinase (HK-IVB) represents the glucose sensor in the beta cell, whereas HK-IVL plays a central role in the regulation of hepatic glucose metabolism.

In human skeletal muscle, HK-II transcription is regulated by insulin, whereas HK-I mRNA and protein levels are not affected by insulin [214–216]. In response to physiologic euglycemic hyperinsulinemia of 2 to 4 hours' duration, HK-II cytosolic activity, protein content, and mRNA levels increase by 50% to 200% in healthy nondiabetic subjects, and this is associated with the translocation of hexokinase II from the cytosol to the mitochondria. In forearm muscle, insulin-stimulated glucose transport (measured with the triple-tracer technique) is markedly impaired in lean type 2 diabetics [207,208], but the rate of intracellular glucose phosphorylation is impaired to an even greater extent, resulting in an increase in the free glucose concentration within the intracellular space that is accessible to glucose. These observations indicate that in type 2 diabetic individuals, although both glucose transport and glucose phosphorylation are severely resistant to the action of insulin, impaired glucose phosphorylation (HK-II) appears to be the rate-limiting step for insulin action. Studies using ^{31}P nuclear magnetic resonance in combination with [1-^{14}C]glucose also have demonstrated that both insulin-stimulated muscle glucose transport and glucose phosphorylation are impaired in type 2 diabetic subjects, but the defect in transport exceeds the defect in phosphorylation [209]. Because of methodologic differences, the results of the triple-tracer technique [207,208] and magnetic resonance imaging [209] studies cannot be reconciled at present. Nonetheless, these studies are consistent in demonstrating that abnormalities in both muscle glucose phosphorylation and glucose transport are well established early in the natural history of type 2 diabetes and cannot be explained by glucose toxicity.

In healthy nondiabetic subjects, a physiologic increase in the plasma insulin concentration for as little as 2 to 4 hours increases muscle HK-II activity, gene transcription, and translation [214]. In lean type 2 diabetics, the ability of insulin to augment HK-II activity and mRNA levels are markedly reduced compared with controls [215]. Decreased basal muscle HK-II activity and mRNA levels and impaired insulin-stimulated HK-II activity in type 2 diabetic subjects have been reported by other investigators [216,217]. A decrease in insulin-stimulated muscle HK-II activity also has been described in subjects with IGT [218]. Several groups have looked for point mutations in the HK-II gene in individuals with type 2 diabetes, and, although several nucleotide substitutions have been found, none are close to the glucose and ATP binding sites and none have been associated with insulin resistance

[218–220]. Thus, an abnormality in the HK-II gene is unlikely to explain the inherited insulin resistance in common variety type 2 diabetes mellitus.

Glycogen synthesis

Following phosphorylation by hexokinase II, glucose either can be converted to glycogen or enter the glycolytic pathway. Of the glucose that enters the glycolytic pathway, approximately 90% is oxidized, and the remaining 10% is released as lactate. At low physiologic plasma insulin concentrations, glycogen synthesis and glucose oxidation contribute equally to glucose disposal; however, with increasing plasma insulin concentrations, glycogen synthesis predominates [1,2,221]. Impaired insulin-stimulated glycogen synthesis is a characteristic finding in all insulin-resistant states, including obesity, IGT, diabetes, and diabesity in all ethnic groups, and accounts for the majority of the defect in insulin-mediated whole-body glucose disposal [1,2,12,98,210,222–224]. Impaired glycogen synthesis also has been documented in the normal-glucose tolerant offspring of two diabetic parents, in the first-degree relatives of type 2 diabetic individuals, and in the normoglycemic twin of a monozygotic twin pair in which the other twin has type 2 diabetes [65,98,225].

Glycogen synthase is the key insulin-regulated enzyme that controls the rate of muscle glycogen synthesis [171,173,216, 226–228]. Insulin activates glycogen synthase by stimulating a cascade of phosphorylation-dephosphorylation reactions (see above discussion of insulin receptor signal transduction), which ultimately lead to the activation of PP1 (also called glycogen synthase phosphatase). The regulatory subunit of PP1 has two serine phosphorylation sites, called site 1 and site 2. Phosphorylation of site 2 by cAMP-dependent protein kinase inactivates PP1, whereas phosphorylation of site 1 by insulin activates PP1, leading to the stimulation of glycogen synthase. Phosphorylation of site 1 of PP1 by insulin in muscle is catalyzed by insulin-stimulated protein kinase (ISPK)-1. Because of their central role in muscle glycogen formation, the three enzymes, glycogen synthase, PP1, and ISPK-1, have been extensively studied in individuals with type 2 diabetes.

Glycogen synthase exists in an active (dephosphorylated) and an inactive (phosphorylated) form [171–173]. Under basal conditions, total glycogen synthase activity in type 2 diabetic subjects is reduced, and the ability of insulin to activate glycogen synthase is severely impaired [185,229–231]. The ability of insulin to stimulate glycogen synthase also is diminished in the normal glucose-tolerant, insulin-resistant relatives of type 2 diabetic individuals [232]. In insulin-resistant nondiabetic and diabetic Pima Indians, activation of muscle PP1 (glycogen synthase phosphatase) by insulin is severely reduced [233]. Because PP1 dephosphorylates glycogen synthase, leading to its activation, a defect in PP1 appears to play an important role in the muscle insulin resistance of type 2 diabetes mellitus.

The effect of insulin on glycogen synthase gene transcription and translation in vivo has been studied extensively. Most studies have demonstrated that insulin does not increase glycogen synthase mRNA or protein expression in human muscle [214,234,235]. Glycogen synthase mRNA and protein levels, however, are decreased in muscle of type 2 diabetic patients, partly explaining the decreased glycogen synthase activity [235,236]. The major abnormality in glycogen synthase regulation in type 2 diabetes is its lack of dephosphorylation and activation by insulin, as a result of insulin receptor signaling abnormalities (see previous discussion).

The glycogen synthase gene has been the subject of intensive investigation, and DNA sequencing has revealed either no mutations or rare nucleotide substitutions that cannot explain the defect in insulin-stimulated glycogen synthase activity [237–239]. The genes encoding the catalytic subunits of PP1 and ISPK-1 have been examined in Pima Indians and Danes with type 2 diabetes [240,241]. Several silent nucleotide substitutions were found in the PP1 and ISPK-1 genes in the Danish population, but the mRNA levels of both genes were normal in skeletal muscle. No structural gene abnormalities in the catalytic subunit of PP1 were detected in Pima Indians. Thus, neither mutations in the PP1 and ISPK-1 genes nor abnormalities in their translation can explain the impaired enzymatic activities of glycogen synthase and PP1 that have been observed in vivo. Similarly, there is no evidence that an alteration in glycogen phosphorylase plays any role in the abnormality in glycogen formation in type 2 diabetes [242].

In summary, glycogen synthase activity is severely impaired in type 2 diabetic individuals, and the molecular cause of the defect most likely is related to impaired insulin signal transduction.

Glycolysis/Glucose oxidation

Glucose oxidation accounts for approximately 90% of total glycolytic flux, whereas anaerobic glycolysis accounts for the other 10%. The two enzymes PFK and PDH play pivotal roles in the regulation of glycolysis and glucose oxidation, respectively. In type 2 diabetic individuals, the glycolytic/glucose oxidative pathway has been shown to be impaired [243]. Although one study [244] has suggested that PFK activity is modestly reduced in muscle biopsies from type 2 diabetic subjects, most evidence indicates that the activity of PFK is normal [230,235]. Insulin has no effect on muscle PFK activity, mRNA levels, or protein content in either nondiabetic or diabetic individuals [235]. PDH is a key insulin-regulated enzyme with activity in muscle that is acutely stimulated by insulin [245]. In type 2 diabetic patients, insulin-stimulated PDH activity has been shown to be decreased in human adipocytes and in skeletal muscle [245,246].

Obesity and type 2 diabetes mellitus are associated with accelerated FFA turnover and oxidation [1,2,12,247], which would be expected, according to the Randle cycle [248], to inhibit PDH activity and consequently glucose

oxidation. Therefore, it is likely that the observed defects in glucose oxidation and PDH activity are acquired secondary to increased FFA oxidation and feedback inhibition of PDH by elevated intracellular levels of acetyl-CoA and reduced availability of NAD. Consistent with this scenario, the rates of basal and insulin-stimulated glucose oxidation are not reduced in the normal glucose-tolerant offspring of two diabetic parents and in the first-degree relatives of type 2 diabetic subjects, whereas it is decreased in overtly diabetic subjects.

Summary

In summary, postbinding defects in insulin action primarily are responsible for the insulin resistance in type 2 diabetes. Diminished insulin binding, when present, is modest and secondary to down-regulation of the insulin receptor by chronic hyperinsulinemia. In type 2 diabetic patients with overt fasting hyperglycemia, a number of postbinding defects have been demonstrated, including reduced insulin receptor tyrosine kinase activity, insulin signal transduction abnormalities, decreased glucose transport, diminished glucose phosphorylation, and impaired glycogen synthase activity. The glycolytic/glucose oxidative pathway is largely intact and, when defects are observed, they appear to be acquired secondary to enhanced FFA/lipid oxidation. From the quantitative standpoint, impaired glycogen synthesis represents the major pathway responsible for the insulin resistance in type 2 diabetes and is present long before the onset of overt diabetes, that is, in normal glucose-tolerant, insulin-resistant prediabetic subjects and in individuals with IGT. Recent studies link the impairment in glycogen synthase activation to a defect in the ability of insulin to phosphorylate IRS-1, causing a reduced association of the p85 subunit of PI 3-kinase with IRS-1 and decreased activation of the enzyme PI3K.

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